REMARKS

Applicants have amended the claims to more particularly define the invention taking into consideration the outstanding Official Action and in an effort to avoid proceeding with an appeal. New claim 40 has been added to the application which is a combination of amended claim 1 and claim 3. Claim 3 has been canceled as redundant. The amendments to the claims are fully supported by the specification as originally filed. Applicants most respectfully submit that all of the claims now present in the application, claims 1, 2, 4 -27 and 31-40, are in full compliance with 35 USC 112 and are clearly patentable over the references of record.

Applicants have amended claim 1 to explicitly state that the synthetic bone material is used in the human or animal body. These limitations are supported by the specification as originally filed as would be interpreted by one of ordinary skill in the art to which the invention pertains. In this regard, the reference to 40,000 hip replacements on page one of the specification would be clearly understood to refer to human patients. The reference to the various known implants on page 2 further supports animal and human patients. It is most respectfully submitted this amendment overcomes the Examiner's comments that the "intended use is very broad" (see the paragraph bridging pages 3 and 4 of the last Office Action).

With regard to the Examiner's comment that the present application does not describe the actual use of the material in-vivo, Applicants submit herewith a copy of a scientific paper by the inventor (Karin Hing et al), which is entitled "Microporosity enhances bioactivity of synthetic bone graft substitutes", Journal of Materials Science: Materials in Medicine 16 (2005) 467-475. It can be seen that this scientific paper specifically refers to the method according to the present invention (see page 468, bottom of first column, and the References, Refs. 17 and 18). The paper presents some positive in-vivo results. As would be appreciated by one of ordinary skill in the art, at

the time of the filing of the present application the material may be used in vivo. The paper describes the actual use of the material in-vivo. The specification as originally filed refers to such a specific use on page 3. It is stated at lines 24-27, that the present invention aims to provide a method for the manufacture of porous material with highly interconnected porosity, which are suitable for use in medical applications.

Moreover, the paper supports Applicants statement that using a ball mill to foam the synthetic bone material enables the independent control of the level of total porosity and the level of strut porosity. Clearly, this paper does support Applicants submission that there is a new/unexpected effect associated with using a ball mill to foam biomedical ceramic materials for use in the human or animal body.

Applicants believe that the important point to stress is that only the present invention has appreciated the advantages associated with ball milling foam-stabilized slips in the production of macroporous ceramic synthetic bone materials for biomedical applications. These advantages are described in the present application (see pages 8, 9, 12 and 21 of the specification) and also in the attached scientific paper. These include:

(i) no organic sponge/foam template or solid pore-formers to burnout; porous ceramics produced by burnout methods often have relatively low mechanical properties resulting from defects in the structure due to incomplete/irregular burnout of the original template;

(ii) homogeneous or functionally graduated pore distributions are attainable by varying the slip viscosity;

(iii) macro-pore size is variable by varying the start-powder particle size;

(iv) macro-porosity is highly interconnected; and

(v) the microstructure contains an interconnected network of micro-pores, the degree of connectivity of which can be controlled during sintering. These advantages enable control of the pore structure so as to minimize batch variation and the production

of substantially isotropic open structures. The claimed processing route therefore enables the structural features, such as the pore size and connectivity, of both the macro-porosity and micro-porosity of the biomedical ceramic material to be tailored to the specific application so that structural and mechanical properties may be matched to particular requirements for use in the human or animal body.

The present application already provides evidence of the technical advantages associated with using a ball mill to foam the synthetic bone material for biomedical applications (see the Examples and the Figures). In particular, the scanning electron micrographs of the biomedical ceramics presented in the Figures show that using a ball mill to foam the synthetic bone material enables the independent control of the level of total porosity and the level of strut porosity. This may be contrasted with the prior art foaming methods for biomedical applications, which do not achieve this level of control. In this regard, Applicants attach an information sheet which provides examples of the prior art foaming methods (e.g. blending, shaking, blowing gas, and gas nucleation) and the typical microstructures of the materials so produced.

With regard to the macroporosity aspect of the microstructure, the conventional method of blending/beating can be seen to result in uni-directional fragmentation of large cells leading to an ellipsoidal pore geometry, which can inhibit the ingress of mesenchymal cells and blood vessels in vivo. The conventional method of shaking can result in multi-directional fragmentation of large cells leading to wide pore size distribution and lower interconnection size, which again can inhibit the ingress of mesenchymal cells and blood vessels in vivo. In the conventional method of blowing gas through a slurry, the pore size is determined by slurry viscosity, nozzle diameter and flow rate. It is often difficult to control pore size distribution due to foam coarsening. The conventional method of gas nucleation can result in a non-uniform and non-interconnected microstructure due to pore coarsening caused by the partial pressure of the blowing agent. Finally, in the conventional method of phase burn-out, entrapment

of carbon can occur in closed pores, and expansion of the sacrificial phase on burn-out often leads to scaffold micro-fracturing.

In view of the foregoing, the present inventors have found that the conventional foaming methods for preparing biomedical ceramics all have their drawbacks. Moreover, the present inventors have found unexpectedly that the application of a ball mill to foam the ceramic slip results in the controlled development of a mono-modal distribution of well interconnected spherical pores and the independent control of the level of total porosity and the level of strut porosity. The present inventors have found that the resulting microstructure results in a synthetic biomedical ceramic having improved properties when used in the human or animal body.

New claim 40 is based on a combination of currently amended claim 1 and dependent claim 3, which recites that the milling media have a diameter in the range of from 10 to 30 mm (and from 15 to 25 mm in claim 34). These ranges are not disclosed in US 5,895,897. While US 5,656,562 does mention 13 mm milling media, this is for grinding the starting powder, not for foaming a ceramic slip. Similarly, while US 5,395,722 does mention 1-30 mm milling media, this is for grinding an organic perylene pigment, not for foaming a ceramic slip. There is no teaching or suggestion in any of the documents that such sized milling media could or should be relied on in the formation of a ceramic foam, let alone a ceramic foam for a synthetic bone material for use in the human or animal body, where the pores have a modal diameter d(mode) of at least 100 microns.

Additionally, on page 8 of the present specification Applicants state that it is preferable that the amount of ceramic particulate when mill-foaming the ceramic slip is from 3 to 20 w/w% ceramic particulate to the milling media (more preferably from 5 to 15 w/w% ceramic particulate to the milling media). This feature is not taught by WO 93/04013, US 5,895,897 or US 5,656,562. While US 5,395,722 does refer to using 1 to 20 parts by weight milling media to 1 part by weight (organic perylene) pigment (see

columns 5, line 68 -Column 6, line 1), Applicants most respectfully submit that this is not relevant to the present case. This is because US 5,395,722 does not use a ball mill to create a ceramic foam. The mill is simply used to grind an organic perylene pigment.

There is also a difference in that the perylene pigment in US 5,395,722 is milled in the absence of any liquid carrier. Thus, it is not appropriate to rely on the disclosed range of 1 to 20 parts by weight milling media to 1 part by weight (organic perylene) pigment because our proposed range would refer to the weight of the ceramic particulate when provided in a liquid carrier. In any case, it would be necessary to combine three separate and completely unrelated documents (i.e. WO 93/04013, US 5,895,897 and US 5,395,722) to even come close to this feature in combination with the features of claim as claimed in claim 40. Moreover, there is absolutely no motivation in the prior art for such a combination. Thus, the combination of references does not render the claimed subject matter prima facie obvious to one of ordinary skill in the art to which the invention pertains. Moreover the resulting properties of the biocompatible material of the present invention are clearly shown as discussed above and the additional publication submitted herewith.

The statement on page 3 of the Final rejection that, "A recitation of the intended use of the claimed invention must result in a structural difference between the claimed invention and the prior art in order to patentably distinguish the claimed invention from the prior art" is specifically traversed. It is the differences in the process of making which results in the difference in properties of the product which results in the patentability of the claimed process. The specific processing steps of the presently claimed invention distinguishes the claimed invention over the prior art.

The decisions cited by the Examiner in support of the Examiner's holding have been carefully considered but it is most respectfully submitted that they do not support the argument presented. Whether a preamble of intended purpose constitutes a limitation to the claims is, as has long been established, a matter to be determined on

the facts of each case in view of the claimed invention as a whole. In re Duva, 387 F.2d 402, 407, 156 USPQ 90, 94 (CCPA 1967); In re Walles, 366 F.2d 786, 790,151 USPQ 185,190 (CCPA 1966). The test in determining whether a claimed invention would have been obvious is what the combined teachings of the references would have suggested to one of ordinary skill in the art. In re Keller, 642 F.2d 413, 425, 208 USPQ 871, 881 (CCPA 198 1). In the present case, the claimed invention is not prima facie obvious for the reasons already of record, herein incorporated by reference and the additional amendments to the claims and information submitted herewith.

The rejection of claims 1, 4 -27, 32,3 and 35-39 under 35 U.S.C. 103(a) as being unpatentable over WO 93/04013 in view of Oishi et al. has been carefully considered but is most respectfully traversed in view of the amendments to the claims and the following comments.

At the outset, Applicants wish to direct the Examiner's attention to the basic requirements of a prima facie case of obviousness as set forth in the MPEP § 2143. This section states that to establish a prima facie case of obviousness, three basic criteria first must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine the reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations.

The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure. In re Vaeck, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991).

Section 2143.03 states that all claim limitations must be taught or suggested by the prior art. In re Royka, 490 F.2d 981, 180 USPQ 580 (CCPA 1974). "All words in a claim must be considered in judging the patentability of that claim against the prior art." In re Wilson, 424 F.2d 1382, 1385, 165 USPQ 494, 496 (CCPA 1970). If an

independent claim is nonobvious under 35 U.S.C. 103, then any claim depending therefrom is nonobvious. In re Fine, 837 F.2d 1071, 5 USPQ2d 1596 (Fed. Cir. 1988).

Applicants most respectfully submit that a prima facie case of obviousness of the presently claimed subject matter has not be established as it is submitted that it would not have been obvious to modify the process described in WO 93/04013 in view of the disclosure of US 5,895,897.

Amended claim 1 is directed to A method of producing a synthetic bone material for use in biomedical applications, said synthetic bone material comprising a macroporous ceramic foam which has an open foam structure containing pores and having an open foam structure containing pores with a modal diameter $d_{mode} \geq 100$ µm.... Thus, the claimed method is concerned with making a synthetic bone material for biomedical applications (see page 1, lines 1 to 5 of Applicants' specification). As now amended, claim 1 specifies that the ceramic particulate is biocompatible.
The term "macroporous" means an open foam structure containing pores with a modal diameter $d_{mode} \geq 100$ µm (see the text on page 4, lines 14 to 17). Claim 1 also now specifies that the synthetic bone material is used in the human or animal body as set forth in step (e) of the method. This obviates the Examiner's comments on page three of the Official Action that the claims do not appear to require using the material as an implant. The claims now also require a use step. These are claim limitations which cannot be ignored.

Claim 1 is further limited in that the step of foaming the ceramic slip in step (b) is carried out using a ball mill.

Claim 1 is novel over WO 93/04013 for at least the reason that this prior art document does not disclose the step of foaming a ceramic slip using a ball mill (step (b)). It is clear that WO 93/04013 achieves foaming by the injection of gas into the dispersion. Claim 1 is further distinguished from WO 93/04013 in that the claimed method is directed to a method of producing a macroporous ceramic foam for use in

biomedical applications and having an open foam structure containing pores with a modal diameter $d_{mode} \geq 100$ µm.

Accordingly, claim 1 differs from WO 93/04013 by virtue of at least the feature of foaming the ceramic slip using a ball mill and also in that the ceramic foam is a macroporous ceramic foam for use in biomedical applications having an open foam structure containing pores with a modal diameter $d_{mode} \geq 100$ µm.

It is further noted that the method according to the primary reference does not appear to result in a porous foamed ceramic structure that would be suitable for use as a biomedical material (e.g. a bone graft substitute) as required by the claims now present in the application. For this purpose, the foamed macroporous ceramic material must exhibit open porosity, as opposed to closed porosity, and must have a modal pore size $\geq$100 µm. This is clearly discussed in the description of the present application (see pages 4 and 5) and reflected by the wording of claim 1. Indeed, the reference to the Buchner funnel in Examples II, III and IV of the primary reference would be expected by one of ordinary skill in the art to result in pores having a similar size to that of the filter, i.e. 10 to 16 µm.

Example VIII teaches a slip of hydroxyapatite wherein the product had a mean pore diameter of 24 µm. Moreover, the primary reference states that the pores may be closed and/or the porosity may be open at page 11, second full paragraph. There is no positive teaching in the primary reference of the open pore structure which is a claim limitation of all of the claims now present in the application. Applicants' specification may not be used as a teaching reference.

Applicants note that the primary reference does refer to gas entrapment by mechanical means and suggests that this may be achieved simply by stirring. This is exemplified in Examples V-X, where a paddle stirrer or stirring in a beaker was used. The other Examples (Example I-IV) rely on a Buchner funnel to produce the foam. It is therefore clear that one of ordinary skill in the art would appreciate that the primary

reference had identified what it considered to be suitable methods for forming a foamed ceramic. There is no indication in the primary reference that there were any problems associated with these foaming methods. Accordingly, there simply would not be any motivation for a person skilled in the art to look elsewhere for an alternative foaming technique.

Claim 1 is further limited in that the step of foaming the ceramic slip in step (b) is carried out using a ball mill. The importance of the properties of the synthetic bone material achieved by ball milling in accordance with the claims on appeal is described in Applicants' specification and is not suggested in the prior art relied upon in the rejection. As discussed on pages 8, 9 and 21 of Applicants' specification, there are a number of advantages associated with ball milling foam-stabilised slips formed in accordance with the presently claimed invention including:

(i) No organic sponge/foam template or solid pore-formers to burnout; porous ceramics produced by burnout methods often have relatively low mechanical properties resulting from defects in the structure due to incomplete/irregular burnout of the original template;

(ii) Homogeneous or functionally graduated pore distributions are attainable by varying the slip viscosity;

(iii) Macro-pore size is variable by varying the start-powder particle size; (iv) Macro-porosity is highly interconnected; and

(iv) Microstructure contains an interconnected network of micro-pores, the degree of connectivity of which can be controlled during sintering. This is important for tailoring the drug delivery characteristics of the porous structure.

These advantages enable control of the pore structure so as to minimize batch variation and the production of substantially isotropic open structures. The claimed processing route therefore enables the structural features, such as the pore size and connectivity, of both the macro-porosity and micro-porosity to be tailored to the specific

application so that structural and mechanical properties may be matched to particular requirements. It is pointed out that all the Examples featured in the present application rely on the use of a ball mill to achieve foaming of the ceramic slip. Thus, the use of a ball mill is a specific aspect of the invention and not simply an equivalent method of foaming the slip.

As noted on page 6 of Applicants' specification the organic binder serves to provide plasticity during forming of the ceramic particulate and green strength in the formed product. It is also noted that all of the examples in Applicants' specification include an organic binder. At page 7, line 5, of Applicants' specification, it is stated that the organic binder will generally be present in a liquid carrier in an amount of from 0.2 to 10 w/v% and more preferably from 0.5 to 6 w/v%. The specific and preferred limitations are specifically set forth in claims 12, 13 and 35 on appeal. There is absolutely no suggestion in the prior art of these specific ranges which are claim limitations. The necessary motivation is not in the prior art to suggest these preferred aspects of the presently claimed invention and for this reason, these claims are further distinguished over the prior art.

The only disclosure in the primary reference to the use of a binder is at page 9 which simply suggests that binders such as resins may be included but there is no suggestion of the specified amounts which are clearly indicated to be preferred embodiments of the presently claimed invention. The examples in the primary reference do not use binders let alone suggest the amounts specified in claims 12, 13 and 35. While the '897 patent describes the use of an organic binder in the paragraph beginning at column 3, line 25, this relates to a foam slurry which is produced by mixing an alumina based ceramic powder, SiC whiskers, and a solution containing a dispersant, an organic binder and a foaming agent in water. This in no way suggests a modification of the primary reference to arrive at the presently claimed preferred binder concentrations as claimed in claims 11, 12 and 35 on appeal.

As discussed at page 20 of Applicants' specification, the results in Table 3 and Figures 7-10 demonstrate how variation in the ratio of ceramic particulate to binder solution variation in both the bulk density (macro-porosity) and the strut density (micro-porosity). The sintered mill-foamed porous ceramics prepared with the greater volume of liquid carrier have lower bulk and strut densities reflecting a more open, inter-connected pore structure with large macro-pores and a larger fraction of micro-porosity.

As noted at page 21, the macro-porous ceramic foams according to the present invention have advantages over the prior art cancellous and coral derived materials. The sintered ceramic foam has a bulk porosity in the range of from 70 to 90% as specifically claimed in claim 37 and a slightly broader range in claim 25. These are specific claim limitations which again are in no way suggested by the prior art. The strut density is specified in claims 26 and 38. Clearly, these limitations are present in the claims, discussed in the specification, and further distinguish the claimed subject matter over the prior art.

Claim 1 differs from the teachings of the primary reference for at least the reasons that this prior art reference does not disclose that the foamed macroporous ceramic material must exhibit open porosity, as opposed to closed porosity, and must have a modal pore size ≥ 100 μm and the step of foaming a ceramic slip using a ball mill (step (b)). It is clear that the primary reference achieves foaming by the injection of gas into the dispersion by either mechanical means e.g. stirring or using a filter of defined pore size, see page five, first paragraph of the primary reference.

In an effort to overcome one of the deficiencies of the primary reference, the Final Rejection relies on the teachings of the '897 patent for foaming by ball milling. However, the '897 patent is directed to a light-weight ceramic acoustic absorber for use in the exhaust nozzles of a jet engine. This reference is directed to a light-weight ceramic acoustic absorber for use in the exhaust nozzles of a jet engine. It is,

accordingly, clear that US 5,895,897 lies in a completely different technical field from that of the present invention, i.e. synthetic bone materials for biomedical applications.

Applicants most respectfully submit that the skilled person, seeking to improve the properties of a ceramic foam for biomedical applications, would not modify the disclosure of WO 93/04013 based on the teaching of US 5,895,897. This absorber has a dense layer provided on the surface of the foamed ceramic, including ceramic fibers as stated at column 2, lines 55-57 which is distinctly different from the structure formed by the process of the present invention as would be appreciated by one of ordinary skill in the art. It is, accordingly, clear that '897 lies in a completely different technical field from that of the present invention, i.e. synthetic bone materials for biomedical applications. Applicants most respectfully submit that the skilled person, seeking to improve the properties of a ceramic foam for biomedical applications, would not modify the disclosure of the primary reference based on the teaching of the '897 patent related to forming an acoustic absorber to obtain the presently claimed invention. In particular, there is no suggestion in either the primary reference or the '897 patent that the use of a ball mill to achieve foaming of a ceramic slip would result in an improved biomedical ceramic material. Accordingly, there would be no motivation for the skilled person to combine the teachings of the primary reference with the '897 patent, absent Applicants' teaching. In re Fritch, 23 USPQ 1780, 1784(Fed Cir. 1992) ("It is impermissible to engage in hindsight reconstruction of the claimed invention, using the applicant's structure as a template and selecting elements from references to fill the gaps.). Moreover, obvious to try is not the standard of obviousness under 35 USC 103(a).

It is further noted that the method according to WO 93/04013 does not appear to result in a porous foamed ceramic structure that would be suitable for use as a biomedical material (e.g. a bone graft substitute) as contemplated by the present application. For this purpose, the foamed macroporous ceramic material must exhibit open porosity, as opposed to closed porosity, and must have a modal pore size $\geq$100

μm. This is clearly discussed in the description of the present application (see pages 4 and 5) and reflected by the wording of claim 1. Indeed, the reference to the Buchner funnel in Examples 2, 3 and 4 of WO 93/04013 would be expected to result in pores having a similar size to that of the filter, i.e. 10 to 16 μm.

As already stated, document US 5,895,897 does not disclose a method of producing a synthetic bone material for use in biomedical applications, e.g. for use as a bone graft substitute. There is also no indication that the ceramic according to US 5,895,897 has an open macroporous structure with a modal pore size ≥ 100 μm, as required by claim 1 of the present application.

Thus, there would be no motivation for one skilled in the art to combine documents WO 93/04013 and US 5,895,897. There is no indication in either documents that ball milling could or should be used to achieve the required macroporous open foam structure, which is necessary for certain biomedical applications. Indeed this feature is clearly precluded by document WO 93/04013.

As stated in MPEP section 2143, the mere fact that references <u>can</u> be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination. *In re Mills*, 916 F.2d 680, 16 USPQ2d 1430 (Fed. Cir. 1990) (Claims were directed to an apparatus for producing an aerated cementitious composition by drawing air into the cementitious composition by driving the output pump at a capacity greater than the feed rate. The prior art reference taught that the feed means can be run at a variable speed, however the court found that this does not require that the output pump be run at the claimed speed so that air is drawn into the mixing chamber and is entrained in the ingredients during operation. Although a prior art device "may be capable of being modified to run the way the apparatus is claimed, there must be a suggestion or motivation in the reference to do so." 916 F.2d at 682, 16 USPQ2d at 1432.).

Even in the unlikely event that the documents were combined, neither document teaches or suggests that foamed macroporous ceramic material has open porosity (as opposed to closed porosity) with a modal pore size ≥ 100 μm. Thus, the combination of references cannot render the claims prima facie obvious.

As discussed in the present application on pages 8, 9 and 21, there are a number of advantages associated with ball milling foam-stabilized slips, including:

(i) No organic sponge/foam template or solid pore-formers to burnout; porous ceramics produced by burnout methods often have relatively low mechanical properties resulting from defects in the structure due to incomplete/irregular burnout of the original template;

(ii) Homogeneous or functionally graduated pore distributions are attainable by varying the slip viscosity;

(iii) Macro-pore size is variable by varying the start-powder particle size; (iv) Macro-porosity is highly interconnected; and

(iv) Microstructure contains an interconnected network of micro-pores, the degree of connectivity of which can be controlled during sintering. This is important for tailoring the drug delivery characteristics of the porous structure.

These advantages enable control of the pore structure so as to minimize batch variation and the production of substantially isotropic open structures. The claimed processing route therefore enables the structural features, such as the pore size and connectivity, of both the macro-porosity and micro-porosity to be tailored to the specific application so that structural and mechanical properties may be matched to particular requirements. It is pointed out that all the Examples featured in the present application rely on the use of a ball mill to achieve foaming of the ceramic slip. Accordingly, it is most respectfully requested that this rejection be withdrawn.

The rejection of claims 2 and 3 under 35 U.S.C. §103(a) as being unpatentable over WO 93/04013 in view of Oishi et al, as applied to claims 1 and 4-27, 32, 33 and 35-

39 above, and further in view of WU has been carefully considered but is most respectfully traversed.

US 5,656,562 relates to a method of improving the properties of ceramic green bodies. While US 5,656,562 does mention the use of a ball mill, this is not used to prepare a foamed ceramic. Instead, the ball mill is merely used to prepare (i.e. mill) the starting powders. This is clear from column 5, lines 31 to 42, were, the powders are milled and then separated from the grinding media. Only then is a slurry formed by adding deionized water. Thus, US 5,656,562 merely describes the conventional technique of using grinding media to mill starting powders. US 5,656,562 is not concerned with foamed ceramics, nor synthetic bone materials for biomedical applications.

The rejection of claims 2 and 3 under 35 U.S.C. §103(a) as being unpatentable over the primary reference in view of the '897 patent as applied to claims 1, 4 -27, 32, 33 and 35-39 above, and further in view of Wu, the '562 patent, is also untenable and should be reversed for the reasons discussed above with respect to the combination of the primary reference and the '897 patent. The '562 patent does not overcome the deficiencies of the combination of references relied upon in the first obviousness rejection.

In the Final Rejection, the Examiner states that '562 patent is cited to teach a conventional size of grinding media. However, claim 2 on appeal is not concerned with milling powders using a grinding media. Instead, claim 2 is concerned with foaming a ceramic slip in a ball mill. This differs from the teaching of the '562 patent in that the starting material is a ceramic slip (not a starting powder) and in that the process produces a foam (not a milled powder). These are fundamental differences as would be appreciated by one of ordinary skill in the art to which the invention pertains. As the Examiner has acknowledged, the '562 patent is not concerned with foamed ceramics, nor synthetic bone materials for biomedical applications.

Applicants note that claim 40 defines that the milling media have a diameter in the range of from 10 to 30 mm. This range is not disclosed in the '897 patent. While the '562 patent does mention 13 mm milling media, this is for grinding the starting powder, not for foaming a ceramic slip. There is no teaching or suggestion in any of the documents that such sized milling media could or should be relied on in the formation of a ceramic foam in accordance with the requirement of claim 3, let alone a ceramic foam for a synthetic bone material, where the pores have a modal diameter as specified by the claims on appeal.

The '562 patent relates to a method of improving the properties of ceramic green bodies. While the '562 patent does mention the use of a ball mill, this is not used to prepare a foamed ceramic. Instead, the ball mill is merely used to prepare (i.e. mill) the starting powders. This is clear from column 5, lines 31 to 42, were, the powders are milled and then separated from the grinding media. Only then is a slurry formed by adding deionized water. Thus, the '562 patent merely describes the conventional technique of using grinding media to mill starting powders. The '562 patent is not concerned with foamed ceramics, nor synthetic bone materials for biomedical applications.

In view of the above comments, it is considered that the disclosure of the '562 patent has been taken out of context and does not establish a prima facie case of obviousness for the claimed subject matter and this rejection should be withdrawn in view of the above comments and further amendments to the claims.

The rejection of claims 2 , 3 and 34 under 35 U.S.C. §103(a) as being unpatentable over the primary reference in view of the '897 patent as applied to claims 1, 4-27, 32, 33 and 35-39, above, and further in view of Nukada et al US 5,395,722, the '722 patent, is also untenable and should be reversed for the reasons discussed above with respect to the combination of the primary reference and the '897 patent. The '722 patent does not overcome the deficiencies of the first obviousness rejection.

With regard to the '722 patent, this reference is even further removed relating as it does to a electrophotographic photoreceptor. Even though the '722 patent does mention the use of a ball mill, this is used to prepare (i.e. mill) an organic perylene pigment. The '722 patent has nothing to do with ceramic powders let alone the preparation of a foamed ceramic bone material for biomedical applications. This reference was located by looking for claimed limitations and then searching the prior art for these limitations. This is improper hindsight reconstruction of the prior art to arrive at the claimed invention. Accordingly, it is most respectfully requested that this rejection be withdrawn.

Applicants believe that the present amendment places the application in condition for allowance. However, if this is not the case, Applicants wish to conduct an interview with the Examiner and would appreciate it very much if the Examiner would be so kind as to contact the undersigned attorney to arrange the interview.

In view of the above comments, further amendments to the claims, and addition documents submitted herewith, favorable reconsideration and allowance of all of the claims now present in the application are most respectfully requested.

Respectfully submitted,

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Microporosity enhances bioactivity of synthetic bone graft substitutes

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This paper describes an investigation into the influence of microporosity on early osseointegration and final bone volume within porous hydroxyapatite (HA) bone graft substitutes (BGS). Four paired grades of BGS were studied, two (HA70-1 and HA70-2) with a nominal total porosity of 70% and two (HA80-1 and HA80-2) with a total-porosity of 80%. Within each of the total-porosity paired grades the nominal volume fraction of microporosity within the HA struts was varied such that the strut porosity of HA70-1 and HA80-1 was 10% while the strut-porosity of HA70-2 and HA80-2 was 20%. Cylindrical specimens, 4.5 mm diameter × 6.5 mm length, were implanted in the femoral condyle of 6 month New Zealand White rabbits and retrieved for histological, histomorphometric, and mechanical analysis at 1, 3, 12 and 24 weeks. Histological observations demonstrated variation in the degree of capillary penetration at 1 week and bone morphology within scaffolds 3–24 weeks. Moreover, histomorphometry demonstrated a significant increase in bone volume within 20% strut-porosity scaffolds at 3 weeks and that the mineral apposition rate within these scaffolds over the 1–2 week period was significantly higher. However, an elevated level of bone volume was only maintained at 24 weeks in HA80-2 and there was no significant difference in bone volume at either 12 or 24 weeks for 70% total-porosity scaffolds. The results of mechanical testing suggested that this disparity in behaviour between 70 and 80% total-porosity scaffolds may have reflected variations in scaffold mechanics and the degree of reinforcement conferred to the bone-BGS composite once fully integrated. Together these results indicate that manipulation of the levels of microporosity within a BGS can be used to accelerate osseointegration and elevate the equilibrium volume of bone.

1. Introduction

Demographic trends and greater expectations regarding quality of life have resulted in an increasing global demand for orthopaedic implants to replace or repair damaged bones & joints [1]. In bone augmentation, auto-grafting is the current 'gold standard' where bone, containing live cells and growth factors, is harvested from the patient. However, autografting has its limitations, the principle ones being the requirement for a second surgical site with associated donor site pain and morbidity, in addition to the obvious limitations in supply. Alternatively allografts (donor bone obtained from a bone bank) are used, however after sterilisation treatments these do not contain the biologic factors and have impaired strength, furthermore supply and bone quality cannot be guaranteed. Porous ceramics have been considered for use as synthetic bone graft substitutes for over 30 years [2], with many reports of a greater degree and faster rate of bone penetration with increasing macroporosity (i.e. pores >50 μm in size) in a wide variety of BGS [3–7]. This phenomenon has been related to both the greater volume available for in-growth and the openness or interconnectivity of the structure. Bone is a mineralised tissue that relies heavily on the presence of an internal blood supply. Any new bone formation or repair must always be preceded by the formation of a vascular network, the rapidity and extent of which is strongly influenced by the degree of structural interconnectivity between pores [8]. This has been elegantly demonstrated by a greater penetration of bone in porous

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implants with larger pore interconnection sizes [7, 9] and improved integration within porous constructs with smaller but well connected porosity as compared to constructs with larger more isolated pores [3].

Furthermore, it is well known that bone is functionally adaptive, i.e. that it responds to external mechanical stimuli to either reduce or increase its mass as required [10]. Mechanical stimuli regulate many cell types, including osteoblasts and osteocytes [11], moreover they have recently been shown to mediate osteoblastic differentiation of osteoprogenitor cells [12]. Therefore, it is possible to see how variation of local strain in scaffold struts as a result of porosity variation may induce or inhibit bone formation. Thus the degree of scaffold porosity could be responsible for regulating the bioactivity of a BGS as a function of its influence on structural permeability [3] (so controlling initial rate of osseointegration) and the local mechanical environment (so mediating the equilibrium volume of new bone within the repair site [4]). However, while it is recognised that both the rate of integration & the volume of regenerated bone may be dependent on features of the macroporosity, recent studies *in vitro & in vivo* have demonstrated biological sensitivity to the level of microporosity within the ceramic struts [13–16]. With evidence to suggest that initially this is through a combination of enhanced angiogenesis [15] and cell adhesion [13, 14], while in the longer term the influence of both micro and macro-porosity on bone adaptation appears to play a role [4, 16].

The aim of this study was to investigate the influence of controlled variations in levels of strut porosity on osseointegration within porous HA (PHA) scaffolds with varied levels of total porosity in order to explore any influence on the rate of early bone formation and the final volume of regenerated bone within the defect site.

2. Materials and methods

Phase pure porous hydroxyapatite (PHA) scaffolds were produced using a novel slip foaming technique which enabled independent control of the level of total porosity and the level of strut porosity [17, 18], where strut porosity is the percentage of microporosity as a fraction of the strut volume, (Fig. 1). Two grades HA70-1 and HA70-2 were prepared with a nominal total-porosity of 70% while a further two grades, HA80-1 and HA80-2 were prepared with a nominal total-porosity of 80% (Fig. 2(a)). Within each of the total-porosity paired grades the nominal volume fraction of microporosity within the HA struts was varied such that the strut-porosity of HA70-1 and HA80-1 was 10% while the strut-porosity of HA70-2 and HA80-2 was 20% (Fig. 2(b)). Phase purity post sintering was verified using X-ray diffractometry (Fig. 3), performed on a Siemens D-5000 X-ray diffractometer in flat plate geometry with Cu-K_α radiation and the X-ray generator operated at 40 kV and 40 mA. Data were acquired from 25–40° 2θ, with a step size of 0.02° at 2.5 s per step and identification of the phases present was achieved by comparing the obtained diffraction patterns with International centre for diffraction data (ICDD) powder diffraction file (PDF) cards [19].

Quantification of both the macro- and microporosity was performed through a combination of immersion densitometry and image analysis of strut porosity from serial sections [20] using a Zeiss Axioskop optical microscope linked to a KS300 image analyser. Immersion densitometry was performed using water on 5 specimens from each batch, each analysed in triplicate. Image analysis was performed on 6 images of 2–3 sections taken from an embedded specimen from each batch. Approximately 10 mm cube specimens were prepared for immersion densitometry and care was taken to remove any surface skin. A minimum size of 15 mm square was used for characterisation of porosity by image analysis, the specimens were vacuum embedded in Epofix resin (Struers, Glasgow) and left to cure at room temperature overnight. Once cured the specimens were polished on an Abramin automatic polisher (Struers, Glasgow) to a 0.04 μm surface finish.

Cylindrical specimens 4.5 mm in diameter & 6.5 mm long were implanted in the cranial, or from an anthropomorphic perspective, 'distal' end of the femur (Fig. 4) of 6 month old New Zealand White rabbits [4] & retrieved for histological, histomorphometric and/or mechanical analysis at 1, 3, 12 and 24 weeks. Specimens for histological/histomorphometric analysis were

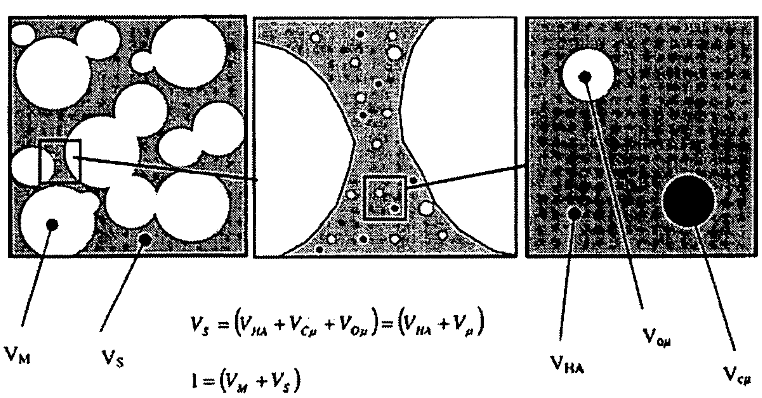

Figure 1 Schematic diagram of the different HA and porosity volume fractions within a porous HA scaffold, where V_S = the total strut volume fraction, V_M = the total macropore volume fraction, $V_{C\mu}$ = the closed micropore volume fraction, V_{HA} = the total HA volume fraction and $V_{O\mu}$ = the open micropore volume fraction.

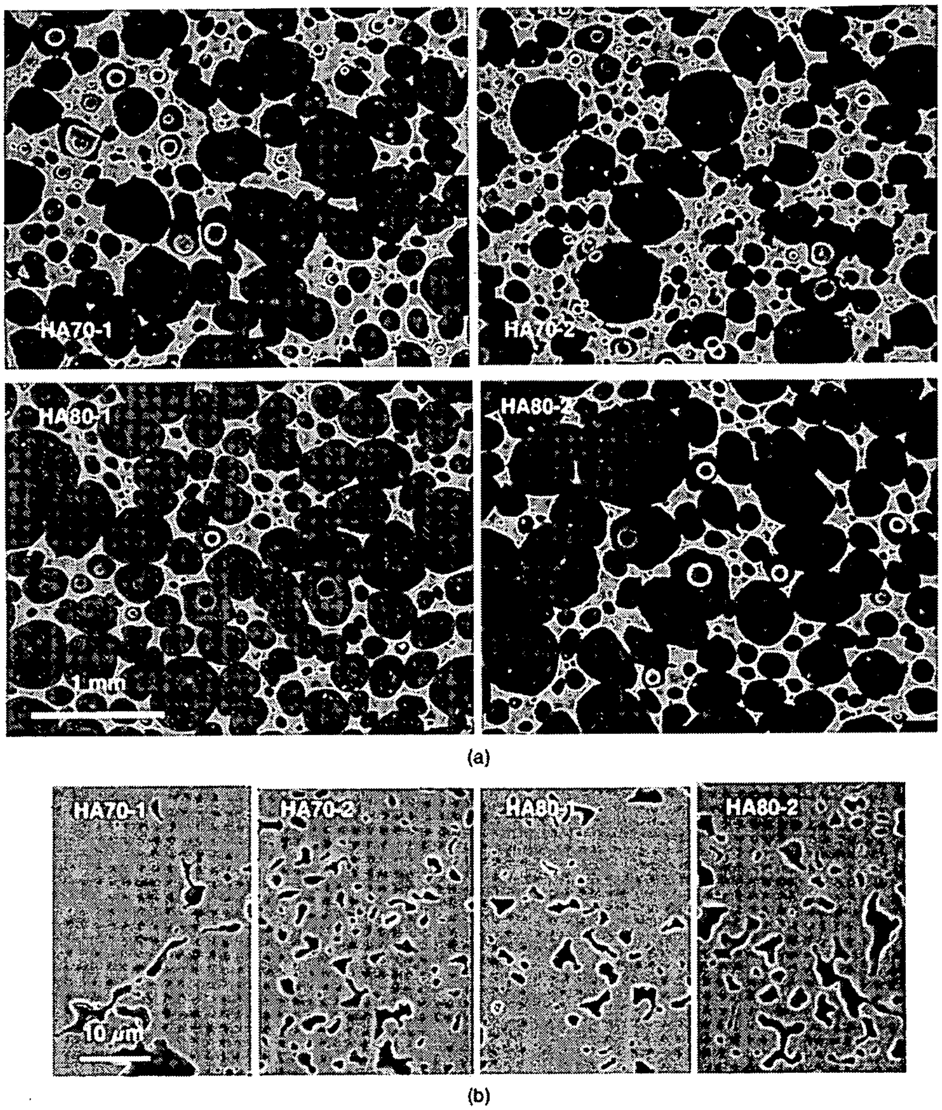

Figure 2 (a) Macropore structures of the four scaffold grades and (b) Microporosity structures of the four scaffold grades.

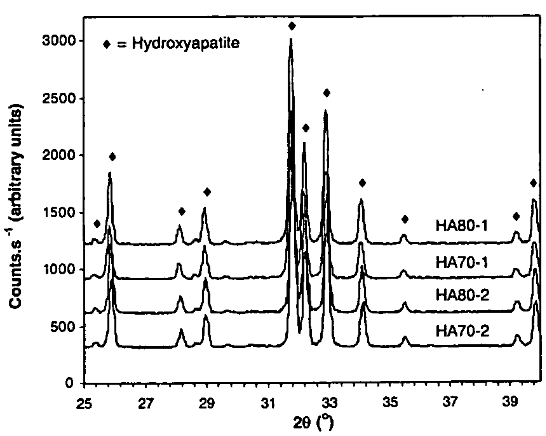

Figure 3 XRD patterns for the four scaffold grades.

trimmed and placed immediately in formal alcohol fixative (comprising 70% ethanol) for a period of at least 4 days. Fixed tissues were dehydrated and embedded in Technovit resin. The resin blocks were then sectioned in the sagittal plane (Fig. 4) and processed through to semi-thin (5–10 μm) section using the Exakt technique [21]. At least three sections were obtained from each implant; one was left unstained for fluorescence microscopy while the others were stained with either toluidine blue or Goldner's trichrome. Histomorphometry was performed using point counting techniques [22] where the space occupied by bone and HA within each implantation site was measured in order to determine the total volumes occupied by bone ingrowth, implant and porosity for each specimen, so enabling calculation of both the absolute and normalised volume% of new bone [4, 23]. The mineral apposition rate (MAR) was determined through administration of fluorochrome labels at 1 & 2 weeks post op to animals allocated to the three week survival group [24]. In order to aid histological and histomophometric examination five regional analysis zones were defined within the implants, and the progression of events such as angiogenesis and new bone formation monitored within these zones (Fig. 4).

Compression testing of both as received cylindrical specimens ($n_{\text{per grade}} = 10$), non-operated control bone

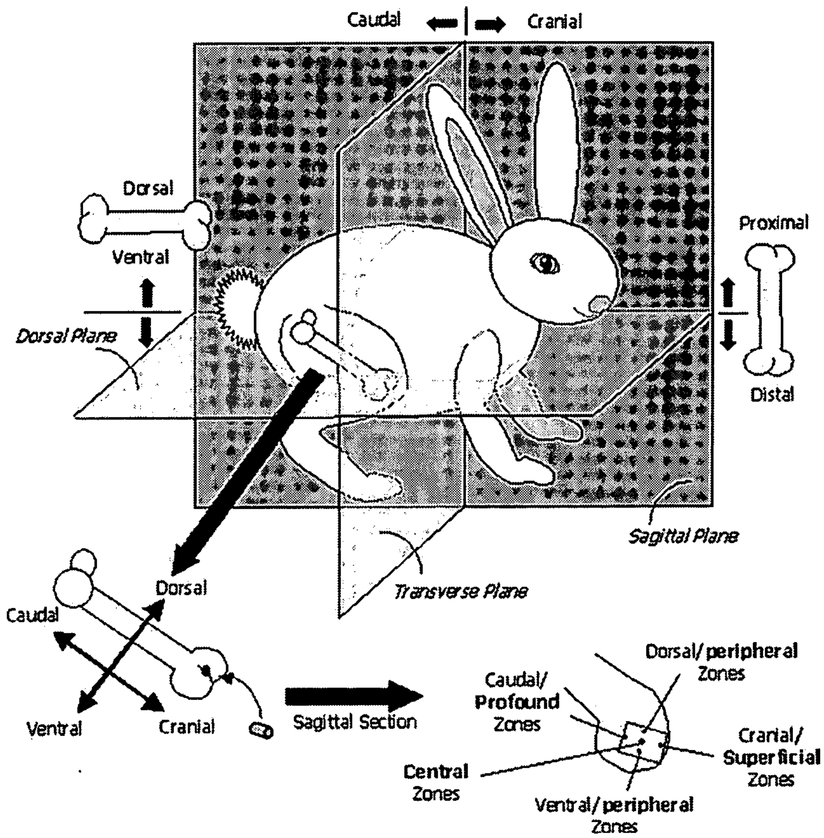

Figure 4 Schematic diagram indicating implantation site and location of analysis zones within a histological section.

($n = 10$) and retrieved implants ($n_{\text{per grade}} = 5$) was performed using an Instron 4464 bench-top test machine, fitted with a 2 kN load cell, using test templates created on Series IX Automated Testing System 1.26 (Instron, High Wycombe, England). Retrieved implants were tested in an environmental chamber at 37 °C. An axial pre-load of 0.005 kN was applied with a crosshead velocity of 0.05 mm s^{-1} prior to application of load to failure with a crosshead velocity of 0.001 mm·s^{-1}. The test was recorded electronically with a sample rate of 0.5 points·s^{-1} [4, 23].

3. Results

Characterisation of the porosity demonstrated that there was no significant difference in either the macroporosity volume fraction or the total porosity of the paired 70 & 80% total-porosity grades (Fig. 5(a)). Furthermore, over 85% of the strut-porosity present within all grades was open & interconnected and was found to vary significantly ($P < 0.05$) between the high and low strut porosity grade paired groups (Fig. 5(b)).

Histological analysis at one week demonstrated that bone ingrowth was of a predominantly woven nature (Fig. 6), and that the primary direction of integration was from the profound zones through to the central zones with some ingress from the peripheral zones. The degree of bone penetration varied with both total-porosity and strut-porosity, within HA80-2, HA80-1 and HA70-2 scaffolds it had reached a depth of 1.0, 1.0–0.5 and 0.5 mm from the profound end, respectively, whereas in HA70-1 scaffolds it was limited to just within the profound & peripheral porosity. Neovascularisation was evident throughout HA80-2, in the central and profound-central porosity of HA70-2 &

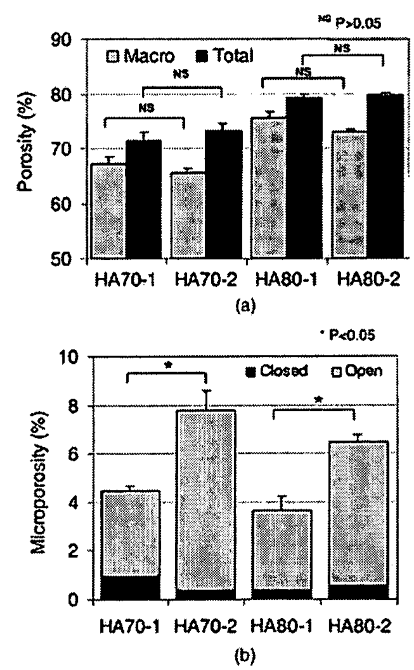

Figure 5 (a) Total and macro- porosity variation within the four scaffold grades and (b) Open and closed microporosity variation within the four scaffold grades.

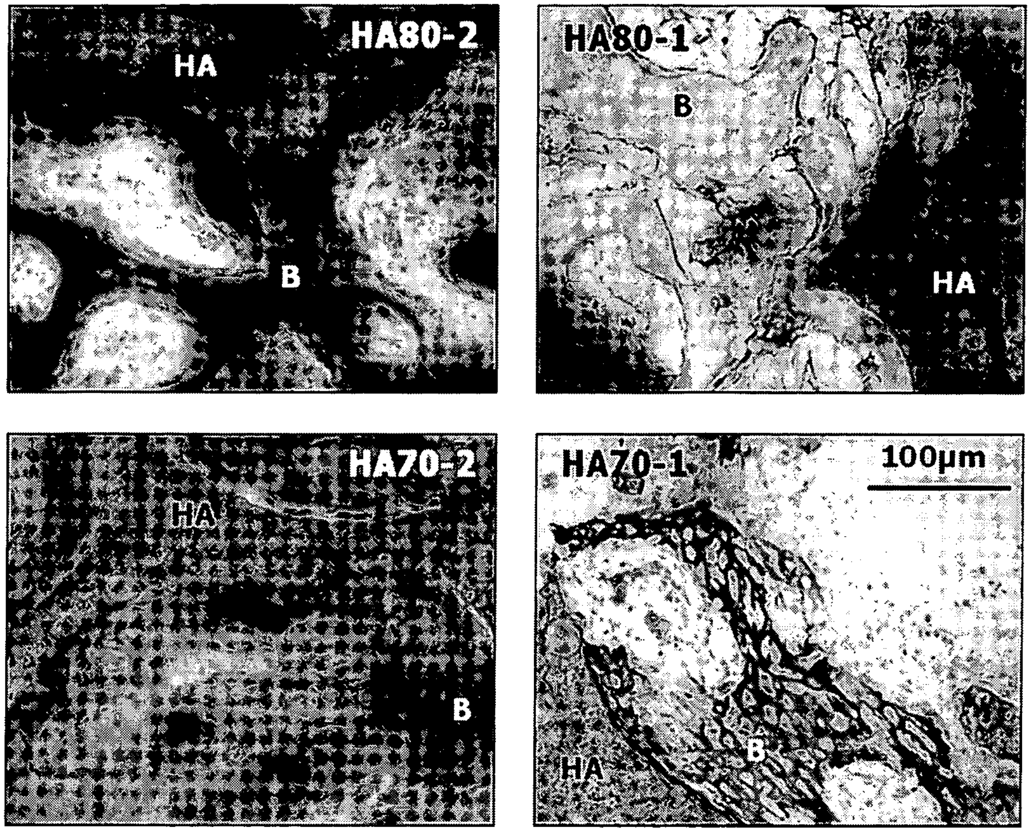

Figure 6 Variation in the morphology of bone found within the 70 and 80% total-porosity paired high and low microporosity BGS scaffolds after 1 week *in vivo*. (HA = BGS strut, B = new bone, HA80-2 & HA70-2 Goldners Trichrome, HA80-1 & HA70-1 Toluidine blue).

HA80-1 respectively, but limited to the profound & peripheral porosity of HA70-1 (Fig. 7). At 3 weeks all new bone was almost exclusively lamellar, however the morphology of bone ingrowth varied with both strut-porosity and total-porosity, appearing denser, i.e. having thicker trabeculae, within the higher microporosity grades, HA70-2 & HA80-2, while ingrowth within the higher total-porosity grades, HA80-1 and HA80-2, was characterised by bone 'islands' in the centre of pores indicative of bone spanning trabeculae, whereas bone within HA70-1 and HA70-2 scaffolds tended to be associated with pore surfaces (Fig. 8). Bone penetration extended throughout the central zones of all scaffolds, with some ingrowth within the superficial zones of HA80-2 scaffolds and limited apposition within the superficial zones of HA70-2 scaffolds. Capillaries were observed throughout HA80-2 and had penetrated to central-superficial zones in HA70-2 and HA80-1 and central zones in HA70-1. There were active regions of bone apposition and resorption (remodelling) through out the profound and central zones of all BGS. By 12 weeks the vascular network was well established in all BGS, marrow had penetrated through out the central zones and the bone had a more mature, organised, lamellar structure however the bone morphology continued to vary within the different porosity BGS, although at this time point the delineation appeared to be between HA80-2 and all other BGS. In HA80-2 bone was characterised by thick pore coverage in combination with trabeculae that spanned pores. There was no significant variation in ingrowth density through out the defect site. Within the other porosity BGS bone morphology varied with location in the defect site, being generally denser in the superficial zone and at anchor points at peripheral porosity. Bone surfaces were often occupied by active osteoblasts or had a scalloped morphology suggestive of regions of active remodelling, particularly in low microporosity BGS (Fig. 9). At 24 weeks there was no significant change in the distribution of bone noted at 12 weeks within the various BGS. The only notable change was in the level of cellular activity, at 24 weeks this was much reduced with few active regions of bone apposition/resorption outside the central zones.

At 3 weeks, despite having similar porosity, there was a significant difference in the absolute % bone ingrowth (%AB) between the total-porosity paired grades of PHA ($^{*}P < 0.05$, Fig. 10(a)) & in the mineral apposition rate (MAR) of bone deposited between weeks 1 & 2 ($^{**}P < 0.005$, Fig. 11). Furthermore, an increase in total-porosity of scaffolds with a strut-porosity of 10% did not significantly affect the volume of ingrowth when normalised for the available pore space (%NB) or the MAR (HA70-1 vs HA80-1 Figs. 10(b) and 11), whereas at 20% strut-porosity there was a significant improvement in both %NB values and MAR with an increase in total-porosity (HA70-2 vs HA80-2 $^{*}P < 0.05$ Figs. 10(b) and 11). In contrast at 12 weeks there were no significant differences in either of the total-porosity paired grades, although the magnitude of bone

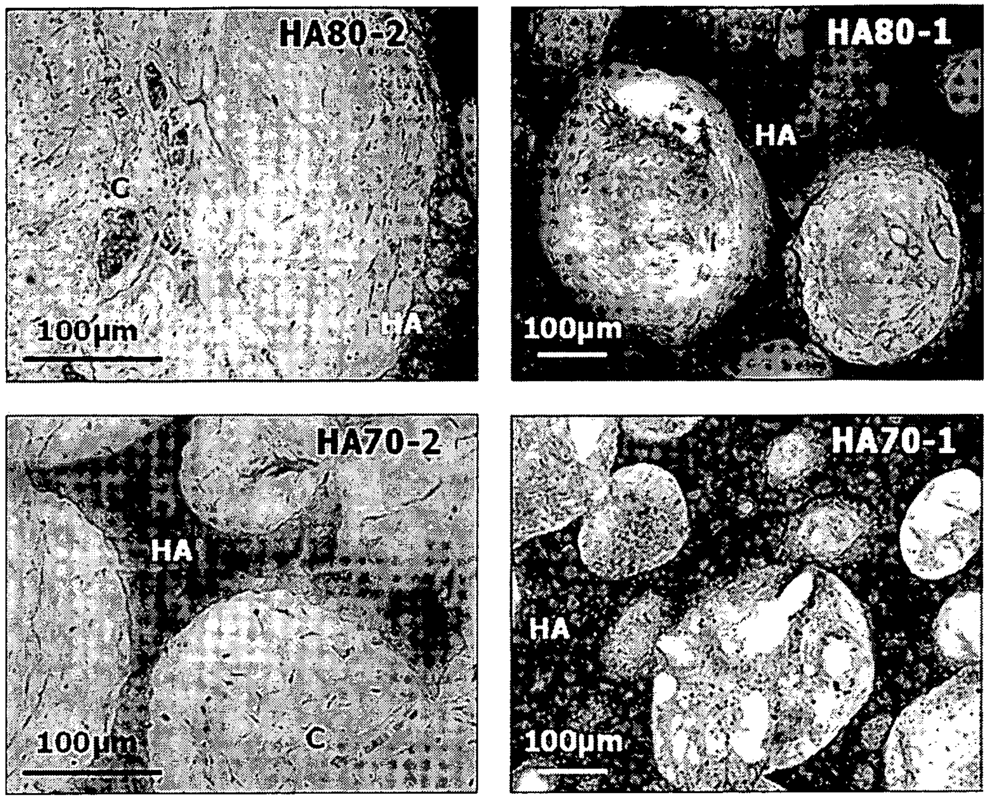

Figure 7 Variation in capillary formation within the central porosity of the 70 and 80% total-porosity paired high and low microporosity BGS scaffolds after 1 week *in vivo*. (HA = BGS strut, C = capillary, HA80-2 & HA70-2 Goldners Trichrome, HA80-1 & HA70-1 Toluidine blue).

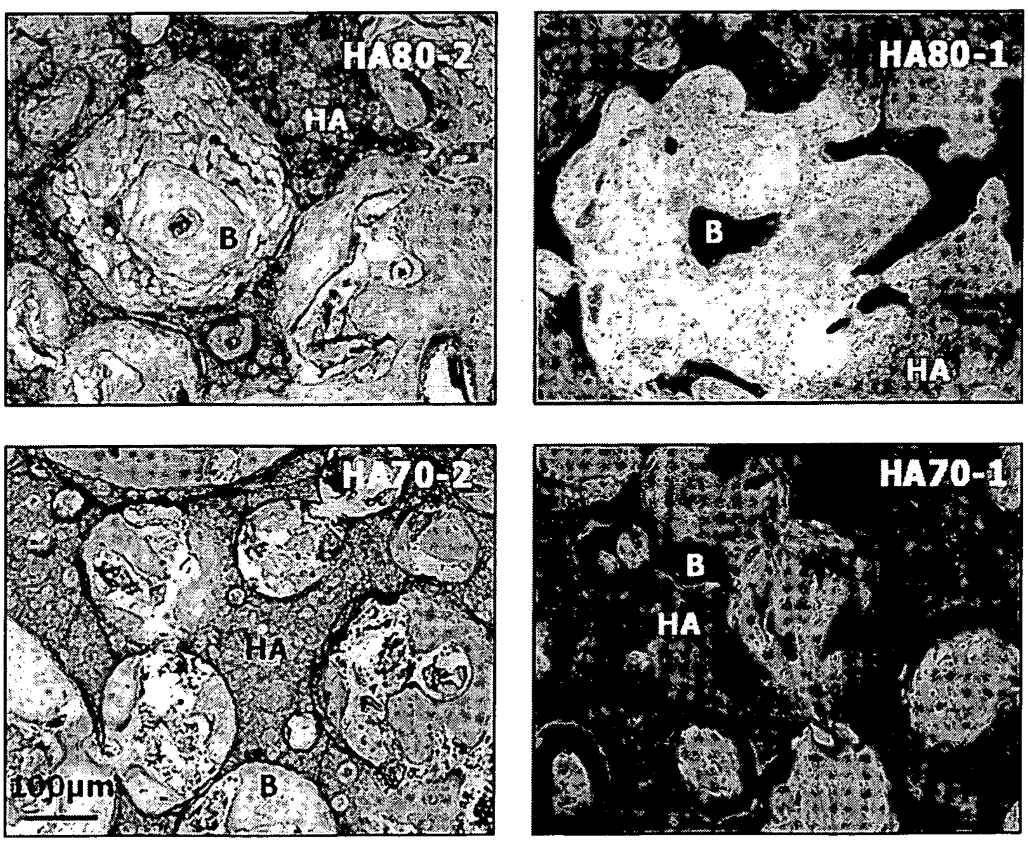

Figure 8 Variation in the morphology of bone found within the central porosity of 70 and 80% total-porosity paired high and low microporosity BGS scaffolds after 3 weeks *in vivo*. (HA = BGS strut, B = bone ingrowth. HA80-2 & HA70-2 Toluidine blue, HA80-1 & HA70-1 Goldners Trichrome).

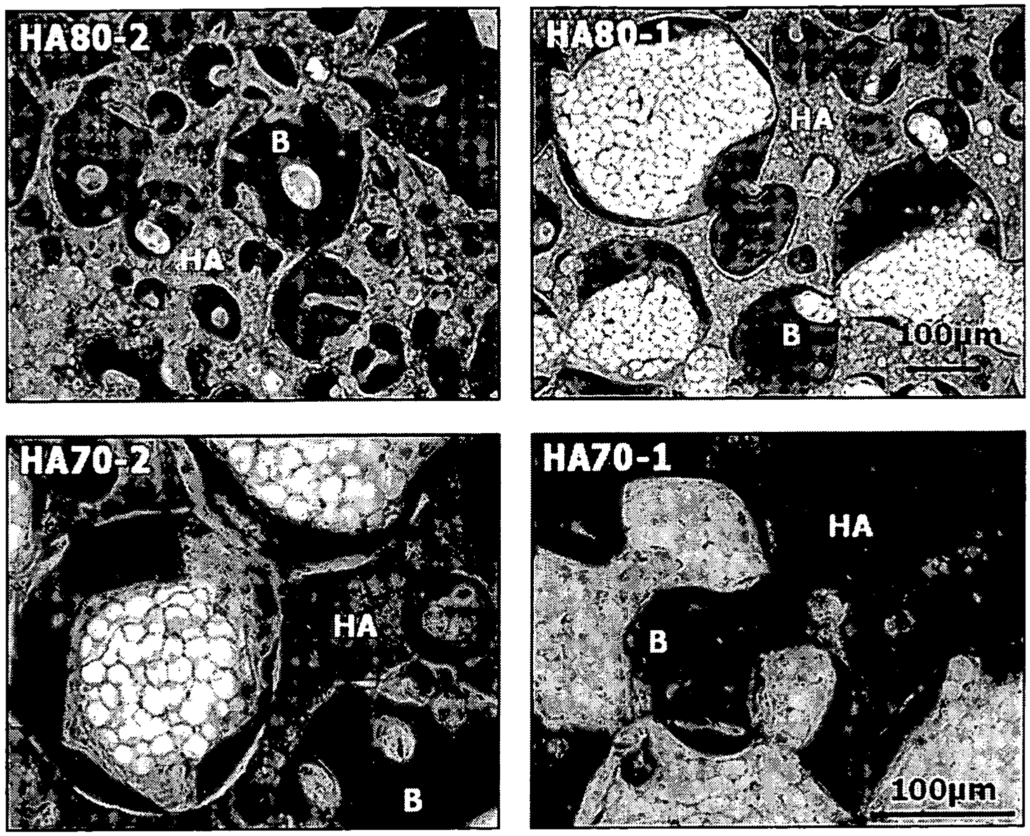

Figure 9 Variation in the morphology of bone found within the central porosity of 70 and 80% total-porosity paired high and low microporosity BGS scaffolds after 12 weeks *in vivo*. (HA = BGS strut, B = bone ingrowth. All Goldners Trichrome).

volume was consistently higher in the high microporosity grades (HA70-2, HA80-2 Fig. 10). By 24 weeks both the %AB and %NB was significantly greater in HA80-2 as compared to HA80-1. In contrast the magnitude of ingrowth in the paired 70% total-porosity BGS was identical. Moreover, the %AB of HA70-1, HA70-2 and HA80-1 was statistically similar despite HA80-1 having a 10% increase in total porosity (Fig. 10).

Prior to implantation the ultimate compressive strengths (UCS) of the as-received BGS varied significantly across the total-porosity paired grades (i.e. HA70-1 vs HA70-2, $P < 0.01$ and HA80-1 vs HA80-2, $P < 0.05$ Fig. 12). However, mechanical testing of the retrieved implants at both 3 and 24 weeks demonstrated the significant reinforcing effect of integration with new bone that resulted in a 300–400% increase in UCS in just 3 weeks (Fig. 12). There was no variation in UCS across total-porosity paired grades at either 3 or 24 weeks, moreover at 24 weeks there was no significant difference in UCS across the entire group of BGS. At 3 weeks the UCS of HA70-1 was significantly greater than that of both 80% total-porosity grades ($P < 0.005$).

4. Discussion

These results indicate that, in PHA scaffolds with equivalent levels of total-porosity, the presence of microporosity altered the pattern and dynamics of osseointegration, where BGS with increased levels of microporosity promoted the apposition of greater volumes of new bone in a more dense morphology at earlier time points. This effect appears to be tied to the rate of vascularisation within the scaffolds, where the disparity in capillary penetration at early time points suggests that the rate of development of the vascular network is linked to the strut porosity variation. Mechanistically, this could be attributed to either increased permeability within the microporous scaffolds enhancing nutrient transfer, leading to faster bone apposition and/or angiogenesis, or it may result from the greater degree of microporosity providing either a larger surface area or a geometrically more suitable substrate for angiogenic and/or osteogenic protein adsorption & cell anchorage, leading to the more rapid induction of angiogenesis and thus bone apposition. This latter hypothesis is supported by previous studies of angiogenesis and osteoblast response *in vivo* and *in vitro*, respectively. Immunohistochemical localisation demonstrated association of VEGF with the HA surface in addition to a close relationship between the HA surface and newly formed capillaries [25] *in vivo*, while an affinity was observed between the fidopillia of primary human osteoblasts-like cells and micropores [13] *in vitro*, respectively.

However, the variation in MAR over the 1–2 week period across the different porosity BGS, would suggest that the alteration in the pattern of osseointegration transpired through a direct increase in the rate of bone formation, suggesting the promotion of early rapid woven bone formation in the more permeable microporous

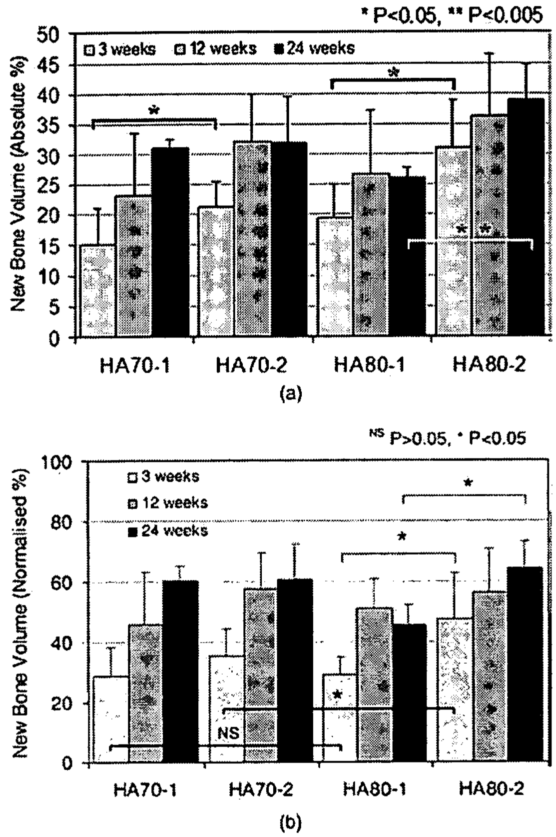

Figure 10 (a) Variation in absolute volume of new bone within the four scaffold grades and (b) Variation in normalised volume of new bone within the four scaffold grades.

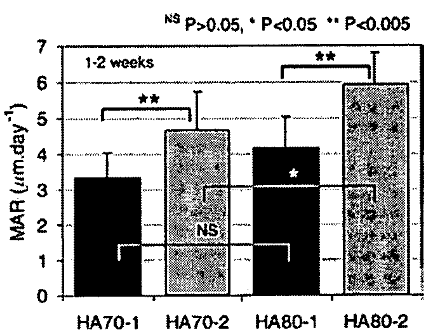

Figure 11 Variation in mineral apposition rate (MAR) of bone between weeks 1-2 within the four scaffold grades.

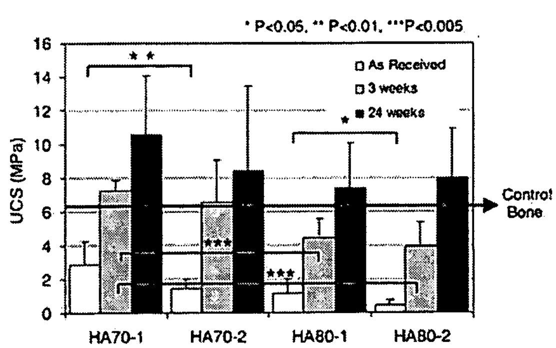

Figure 12 Variation in ultimate compressive strength (UCS) of the four scaffold grades before implantation and after 3 and 24 weeks *in vivo*.

scaffolds (which may not necessarily be linked to the rate of angiogenesis). However, no difference was observed in the organisational maturity of the labelled bone across the four porosity grades, moreover a significant portion of the bone labelled at both 1 and 2 weeks was lamellar. Woven bone, where it occurred, tended to be found largely within the peripheral zones. Taken with the histological observations of predominantly woven bone formation in 1 week survival sections, this predominance of lamellar bone formation from 1–2 weeks would suggest that rapid woven apposition was limited to the periphery during fixation of the implant, with more ordered bone formation dominating after stabilisation of the wound site. Thus the similarity in organisational maturity of bone within the porosity would suggest that the variation in rate within the scaffolds did not correlate to switching between predominantly lamellar or woven modes of bone formation within regions of the scaffold as vascular penetration progressed, but that beyond the first week post-implantation and establishment of graft fixation, the scaffolds supported the direct apposition of lamellar bone, the rate of formation of which was tied to the local conditions of oxygenation and/or the concentration of osteogenic factors at the graft surface. A more detailed examination of the dynamics of apposition in the 2–6 week period will be necessary to identify whether this alteration in the rate of bone formation reflects a gross acceleration of lamellar mineral apposition rate in more microporous scaffolds or whether the increase in microporosity has lead to faster angiogenesis and a temporal shift in the healing cascade.

The alteration in the early pattern of osseointegration and variation in apposition rate only resulted in a significant difference in bone volume between both the 70 and 80% total porosity paired grades at 3 weeks. Furthermore, ultimate levels of bone volume were only significantly different at 24 weeks between HA80-1 and HA80-2. Thus a 10% increase in total porosity had no significant effect on final bone volume for low microporosity BGS (i.e. between HA70-1 and HA80-1). This implies that increasing the microporosity had an additive affect on the absolute equilibrium level of bone established within the macroporosity.

Measurement of the UCS of retrieved implants at 3 weeks demonstrated the extent of reinforcement conferred to the BGS by the new bone, even when partially integrated. Of note was the lack of significant variation in UCS between the total-porosity paired grades, especially given the significant variation in their bone volumes at this time point. Moreover, at 24 weeks there was no significant difference in the UCS of the retrieved implants irrespective of their original porosity and hence strength, again despite the significantly greater level of ingrowth in HA80-2. Taken together with the variation in bone morphology within the four grades of BGS at 12 and 24 weeks (where bone ingrowth remains uniformly dense throughout HA80-2, but varies with location in the other grades) the uniformity in UCS at 24 weeks implies that both bone volume and morphology within BGS may be mechanically mediated at later time points. This phenomenon of bone adaptation within PHA has

previously been reported [4, 16], moreover, remodelling within normal bone is believed to be in response to micro-fractures and changes in the mechanical environment occurring within the bone [26–28]. Thus it is likely that the reduction in scaffold modulus associated with increasing the strut porosity in HA80-20 scaffolds shifted the strut modulus below a threshold value resulting in a swing in the equilibrium local bone cell activity towards a greater degree of stable bone apposition. Interestingly, the absolute equilibrium level of bone established within the macroporosity of HA70-1 was statistically similar to that within HA70-2 and HA80-1 at 24 weeks, suggesting that in order to attain a significant reduction in the equilibrium level of bone the scaffold modulus may require increasing beyond a further threshold value than that of HA70-1. Moreover, once integrated none of the implants were significantly different to control bone from the same site retrieved and tested under identical conditions.

5. Conclusions

The distribution of porosity volume between the macro- and micro- structure had a significant effect on the early pattern and dynamics of osseointegration, possibly though its influence on permeability and angiogenesis, where an increase in either total- or strut-porosity accelerated osseointegration.

In the longer term the dominating factor was the influence of strut-porosity on the mechanics of the scaffold, where the variation in bone ingrowth coupled with the consistency in UCS across the BGS grades at 24 weeks supported the hypothesis that mechano–transduction was the dominant mechanism controlling ultimate bone volume and morphology. This phenomenon resulted in scaffolds with a significantly lower level of total porosity (HA70-2 & HA70-1) having an equivalent level of ultimate bone volume as the 'more porous' HA80-1.

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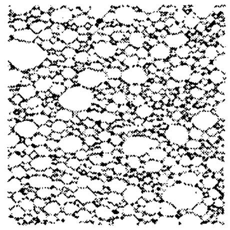
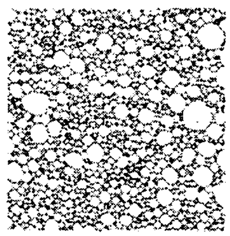
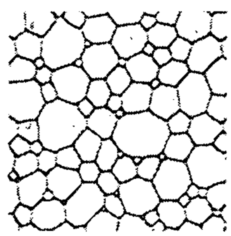
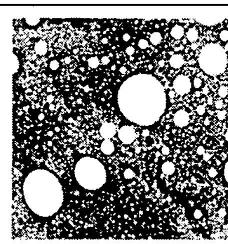
Method	Microstructure	Comment
Blending	 Blending	Uni-directional fragmentation of large cells leading to an ellipsoidal pore geometry [b] which could inhibit the ingress of mesenchymal cells and blood vessels *in vivo* [c]
Shaking	 Shaking	Multi-directional fragmentation of large cells leading to wide pore size distribution and lower interconnection size which could inhibit the ingress of mesenchymal cells and blood vessels *in vivo* [c]
Blowing Gas	 Blowing gas (turbulent flow)	Pore size determined by slurry viscosity, nozzle diameter and flow rate. Difficult to control pore size distribution due to foam coarsening
Gas Nucleation	 Nucleation of gas	Non uniform and not interconnected due to pore coarsening caused by partial pressure of blowing agent